hereinafter Huet.). Please cancel claims 3, 4, 5 and 7 without prejudice. Independent claim 1 and dependent claims 2, 6, 10, 11, 12, 15 and 20 have been amended.

The claims before the examiner are directed to a recombinant potyvirus construct and use thereof.

§ 112 second paragraphs rejections

The Examiner has correctly pointed out that claims 1-7, 10-12 and 20 recite a "substitution" which is indefinite because no reference point is provided. The Examiner further queries amino acid name and number and whether the substition is an amino acid substitution. The Applicant, in order to eliminate these ambiguities, and in order to expedite prosecution, has amended claim 1 to include the limit :

> "...the construct comprising a full length clone characterized by a single mutation, said single mutation residing in its HC- Pro gene conserved FRNK box encoding sequence said single mutation encoding a substitution of Arg by Ile..."

in place of the word "subsitution".

Support for this amendment is found in claims 3, 4 and 5, now cancelled. Therefore, this amendement does not constitute an introduction of new matter and should not require a new examination.

The Applicant respectfully asserts that one of ordinary skill in the art of molecular virology and having reasonable familiarity with potyviridae would sufficiently comprehend the metes and bounds of the claimed invention from claim 1 as currently amended. In support of this assertion, the Applicant refers the Examiner to the prior art of Huet which is of record in this case. In view of the explanation of the phrase "FRNK box" by Huet and others, a reference to the numerical position of the Arg to Ile mutation tn the claim would render the claim ambiguous. This is because of slight differences in position of the FRNK box in various

species of potyviridae. The Applicant thanks the examiner for pointing out the ambiguity of the claims as originally filed.

With regard to claim 15, the phrase "full length clone" which lacks antecedent basis, has been amended to read -- recombinant potyvirus infectious nucleic acid construct--.

With regard to claim 20, the Examiner has asserted that the phrase "collecting the results progeny" is unclear. The Applicant does not find this phrase in claim 20 as filed and respectfully inquire whether the examiner may have been referring to claim 16 which includes this phrase. Assuming that is the case, claim 16 is not currently under examination and the Examiner's Rejection is rendered moot.

All rejections under § 112 second paragraph are moot in view of the claims as currently amended.

§ 112 first paragraph rejections; written description

Claims 7 and 10 stand rejected under 35 USC § 112, first paragraph (written description).

With respect to claims 7 and 10, the Examiner has asserted that they contain subject matter which was not described in the specification in a way which conveys to one of ordinary skill in the art that the inventors had possession of the invention at the time of filing. Specifically, the Examiner refers to a specific strain of virus ZYMV-AG1. Applicant responds by canceling claim 7 and amending claim 10 so that it no longer depends from claim 7. These actions render the Examiner's rejection of claims 7 and 10 under 35 USC § 112, first paragraph (written description) moot.

§ 112 first paragraph rejections; enablement

Claims 1-7, 10, 11, 12, 15 and 20 stand rejected under 35 USC § 112, first paragraph (written description).

With respect to claim 1, the Examiner asserts that "a recombinant potyvirus infectious nucleic acid construct useful for plant cross protection, characterized only in that its HC-Pro gene conserved FRNK box contains a substitution" would require undue experimentation since sufficient guidance as to what substitution to make is not provided. As the Examiner points out, the specification teaches a ZYMV-AG1 construct containing an Arg to Ile substitution in the FRNK box. Therefore, in order to expedite prosecution, the applicant has amended claim 1 to read:

> A recombinant potyvirus infectious nucleic acid construct useful for plant cross protection, the construct comprising a full length clone characterized by a single mutation, said single mutation residing in its HC- Pro gene conserved FRNK box encoding sequence said single mutation encoding a substitution of Arg by Ile;
> wherein the construct is capable of systemic infection of a plant;
> wherein said systemic infection induces a mild form of disease; and
> wherein said systemic infection affords cross protection against a subsequent potyvirus infection.

The Applicant stresses that the amendment to claim 1 does not constitute an introduction of new matter as the limits introduced into the claim were previously present in claims 3, 4 and 5, now cancelled. The applicant respectfully asserts that the detailed explanation of where the substitution is made, how it is effected and what effect it has on viral pathogenicity is found in the specification as filed (PCT IL/99/00184; page10 last paragraph-page 11 first complete paragraph; also figure 1 and tables 1 and 2):

> "Previously, sequence comparison has shown four amino acid changes in the 455 amino acid sequence of the HC - pro gene between the severe field strain (ZYMV - JV) and the mild field strain ZYMK - WK. The replacement of a fragment of the HC - Pro of ZYMV - WK containing two substitutions Aspartate (Asp) 148 and Arg 180 (BstXI/BstEII fragment), reduced

symptom expression of the virus in squash plants without effecting virus accumulation. To distinguish which of the two substitutions, Asp 148 or Arg 180, effect symptom development, Arg 180 was replaced by Ile within the FRNK box (figure 1, clone d) by site directed mutagensis.
The engineered virus containing the Arg 180 replacement by Ile, was designated ZYMV-AG1. This new strain did not cause the development of symptoms in cucumber (three different varieties), melon and watermelon. The virus did accumulate to levels as high as that of the wild type ZYMV-JV. It was assumed, therefore, that the second amino acid difference (Asp at position 148) is dispensable for altering the symptoms from mild to severe.
In order to verify the presence of the amino acid changes within the mild virus" and

(PCT IL/99/00184; page 9 first paragraph):

Example 1 - full length clone (FLC) of ZYMV

Construction of the mutants in the full length clone (FLC) of ZYMV
The constructs which represent the HC - Pro sequences (Huet H., Gal On A., Meiri E., Lecoq H. and Raccah B.(1994) *Journal of General Virology* 75:1407-1414) of the ZYMV - WK strain were placed under the T7 RNA promoter in the infectious FLC. In order to get higher rate of infection with those constructs the fragment BstXI/AgeI from the FLC of 35SZYMVNOS cDNA (Gal On A., Antignus Y., Rosner A., and Raccah B. (1991) *Journal of General Virology* 72:2639-2643 and Gal On A., Meiri E., Huet H., Hua W.J., Raccah B. and Gaba V. (1995) *Journal of General Virology* 76:3223-3227), was replaced by the appropriate fragment from pZYHC (-) clone (Huet H., Gal On A., Meiri E., Lecoq H. and Raccah B.(1994) *Journal of General Virology* 75:1407-1414). Site directed mutagenesis was introduced on ssDNA template of the subclone pksM16B (Gal On A., Antignus Y., Rosner A., and Raccah B. (1991) *Journal of General Virology* 72:2639-2643), using the primer 5' ATGTTCATAAATAAGCGCTCTAG3' (amino acid Ile is underlined and the unique restriction site of Eco47III is in bold). The clone pksM16B carrying the mutations was double digested by BamHI/BstEII and the obtained fragment (1.4kb) was introduced to the same sites in the 35SZYMVNOS cDNA

(Gal On A., Meiri E., Huet H., Hua W.J., Raccah B. and Gaba V. (1995) *Journal of General Virology* 76:3223-3227).

The Examiner states that "This substitution can be any substitution". That assertion is not valid in view of the amendment currently entered. Further, the Examiner states that "Applicant teaches an isoleucine substitution for arginine but lacks a showing of criticality for any of the other amino acids." Applicant points to conservation of the FRNK box among potyviridae as evidence of the criticality of the sequence. Applicant further argues that it is appropriate to formulate claims based on the available experimental data (enablement). The claims, once formulated, delimit the metes and bounds of the invention. Others are free to claim other mutations in the FRNK box if those amino acids are not critical to virus function.

The Examiner's rejection based on use of the word "substitution" is rendered moot by the amendment to claim 1 as described hereinabove.

The Applicant respectfully asserts that the claims as currently amended and the specification as filed provide guidance as to how to perform a substitution in a way which will produce a predictable result. Further, the applicant argues that no undue experimentation would be required to make or use the instantly claimed invention.

Applicant respectfully asserts that the specification as filed enables one of ordinary skill in the art to make and use the invention as instantly claimed.

All §112 rejections are traversed.

§ 102 (b) rejections- anticipation byHuet

Claims 1-6, 10, 11, 12, 15 and 20 are rejected under 35 USC § 102(b) as being directly anticipated by Huet *et al.* (J. General virology 75:1407-1414; hereinafter Huet.).

The examiner asserts that Huet teaches a recombinant potyvirus infectious nucleic acid construct with an Arg to Ile substitution in the FRNK box citing table 2, p1410.

However, claim 1 as currently amended is directed to a construct "...a full length clone characterized by a single mutation...). The word single means one only; one and no more; individual (Websters's New Word Dictionary 2nd College Edition; 1976, D. Guralnik ed.). Therefore, ZYMV-HC (Huet, table 2, p1410) does not anticipate the invention of claim 1 as currently amended.

Further, Huet contains neither a hint nor suggestion that the Arg to Ile substitution in the FRNK box attenuates symptoms of viral infection without interfereing with infectivity as instantly claimed (Claim 1):

> "...said single mutation encoding a substitution of Arg by Ile;
> wherein the construct is capable of systemic infection of a plant;
> wherein said systemic infection induces a mild form of disease; and wherein said systemic infection affords cross protection against a subsequent potyvirus infection."

Further Huet neither hints nor suggests that cross protection is correlated to infection with a construct as instantly claimed.

Respectfully submitted,

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Date: April 28, 2003

AMENDMENTS TO THE CLAIMS
Marked up version showing changes entered as part of the current response

Please cancel claims 3, 4, 5 and 7.

1) (Amended) A recombinant potyvirus infectious nucleic acid construct useful for plant cross protection, the construct comprising a full length clone characterized [only in that] by a single mutation, said single mutation residing in its HC- Pro gene conserved FRNK box encoding sequence [contains a substitution] said single mutation encoding a substitution of Arg by Ile;
wherein the construct is capable of systemic infection of a plant;
wherein said systemic infection induces a mild form of disease; and wherein said systemic infection affords cross protection against a subsequent potyvirus infection.

2) (Amended) A recombinant construct according to claim 1 wherein the nucleic acid is cDNA or an RNA transcript.

6) (Amended) A recombinant potyvirus infectious nucleic acid construct according to [claim 1-5] any of claims 1 and 2 wherein the potyvirus is ZYMV.

10) (Amended) A recombinant construct according to claim [7,8 and 9] 6 [useful for plant cross protection wherein the] wherein said cross protection is against severe strains of ZYMV.

11) (Amended) A recombinant potyvirus infectious nucleic acid construct according to any of claims [1-6] 1, 2 and 6 wherein the potyvirus is selected from BCMV, BYMV, BtMV, MWMV, OYDV, PRSV, PStV, PepMoV, PVMV, CGVBV,

GEV, ISMV, JGMV, LYSV, LMV, MDMV, PPV, PVA, PVV , PVY, SCMV, SPFMV, TEV, TVMV, TBV, TuMV, WMV-2 , YMV and ZYFV.

12) (Amended) A recombinant construct according to any of claims [1-11] 1, 2, 6, 10 and 11 further useful for the transient expression of foreign nucleic acid in plants wherein the full length clone has, in any position, a sequence of DNA or RNA inserted into the full length clone.

15) (Amended) A method for introducing foreign nucleic acid into plants comprising infecting a plant with a [full length clone] recombinant potyvirus infectious nucleic acid construct as defined in claim 11.

20) (Amended) Compositions for plant inoculation or for transient expression of foreign nucleic acid in plants containing, as an active ingredient, the recombinant construct according to any of claim [1-12] 1, 2, 6, 10, 11 and 12. [or a virus containing the recombinant construct according to claim 17.]